

WHAT IS CLAIMED IS:

1. A method of producing a high mannose glycoprotein comprising
  - a. introducing and expressing a polynucleotide encoding a glycoprotein into a mammalian cell;
  - 5       b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
  - c. isolating the lectin resistant mammalian cell;
  - d. culturing said lectin resistant mammalian cell in the presence of deoxymannojirimycin and kifunensine in an amount and for a time to  
10       inhibit glycosylation of the glycoprotein; and
  - e. collecting the high mannose glycoprotein.
2. The method of Claim 1, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.
- 15   3. The method of Claim 2, wherein said lectin is ricin.
4. The method of Claim 1, wherein said glycoprotein is a lysosomal hydrolase.
5. The method of Claim 4, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$  -L-iduronidase,  $\alpha$  -galactosidase A, arylsulfatase , N-acetylgalactosamine-6-sulfatase or  $\beta$  -galactosidase, iduronate 2-  
20       sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside,

Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub> Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

6. The method of Claim 5, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.
7. The method of Claim 1, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.
8. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2.
9. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.
10. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NOS:4, 5 and 7.
11. The method of Claim 7, wherein the GlcNAc-phosphotransferase is encoded by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:1.
12. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises an  $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit, which is encoded by a

nucleotide sequence comprising SEQ ID NO:6 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6.

13. The method of Claim 7, further comprising purifying said glycoprotein after said contacting.

5 14. The method of Claim 7, wherein after said contacting with GlcNAc-phosphotransferase the method further comprises contacting with said glycoprotein with a phosphodiester  $\alpha$ -GlcNAcase.

15. The method of Claim 14, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises an amino acid sequence of SEQ ID NO:18.

10 16. The method of Claim 14, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17.

17. The method of Claim 14, further comprising purifying said glycoprotein after said contacting.

15 18. The method of Claim 1, wherein said deoxymannojirimycin is present in an amount from about 0.1 mM to about 5.0mM.

19. The method of Claim 1, wherein said kifunensine is in present in an amount from about 0.1  $\mu$ g/ml to about 10 $\mu$ g/ml.

20. A high mannose glycoprotein produced by the method of Claim 1.

20 21. A method of producing a high mannose glycoprotein comprising

- a. culturing a lectin resistant mammalian cell in the presence of deoxymannojirimycin and kifunensine in an amount and for a time to inhibit glycosylation of the glycoprotein; and

b. collecting the high mannose glycoprotein.

22. The method of Claim 21, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

5 23. The method of Claim 22, wherein said lectin is ricin.

24. The method of Claim 21, wherein said glycoprotein is a lysosomal hydrolase.

25. The method of Claim 24, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub> Galglucoside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

26. The method of Claim 25, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

20 27. The method of Claim 21, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.

28. The method of Claim 27, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2.

29. The method of Claim 27, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NO:2 and SEQ ID NO:7.

30. The method of Claim 27, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NOS:4, 5 and 7.

5 31. The method of Claim 27, wherein the GlcNAc-phosphotransferase is encoded by  
a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence that  
hybridizes under stringent conditions to the complement of SEQ ID NO:1.

32. The method of Claim 27, wherein the GlcNAc-phosphotransferase comprises an  
 $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence  
10 comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under stringent  
conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit, which is encoded  
by a nucleotide sequence comprising SEQ ID NO:6 or a nucleotide sequence that  
hybridizes under stringent conditions to the complement of SEQ ID NO:6.

33. The method of Claim 27, further comprising purifying said glycoprotein after said  
15 contacting.

34. The method of Claim 27, wherein after said contacting with GlcNAc-  
phosphotransferase the method further comprises contacting with said  
glycoprotein with a phosphodiester  $\alpha$ -GlcNAcase.

35. The method of Claim 34, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises  
20 an amino acid sequence of SEQ ID NO:18.

36. The method of Claim 34, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17.
37. The method of Claim 34, further comprising purifying said glycoprotein after said contacting.
38. The method of Claim 21, wherein said deoxymannojirimycin is present in an amount from about 0.1 mM to about 5.0mM.
39. The method of Claim 21, wherein said kifunensine is in present in an amount from about 0.1  $\mu$ g/ml to about 10 $\mu$ g/ml.
40. A high mannose glycoprotein produced by the method of Claim 1.
41. A method of treating a patient suffering from a lysosomal storage disease comprising administering to said patient a lysosomal hydrolase in an amount sufficient to treat said disease, wherein said lysosomal hydrolase is obtained by a method comprising:
- a. culturing a lectin resistant mammalian cell in the presence of deoxymannojirimycin and kifunensine in an amount and for a time to inhibit glycosylation of the glycoprotein;
  - b. collecting the high mannose glycoprotein;
  - c. collecting the lysosomal hydrolase from said lectin resistant cells;
  - d. contacting the collected lysosomal hydrolase with a GlcNAc-phosphotransferase; and
  - e. contacting said lysosomal hydrolase with a phosphodiester  $\alpha$  GlcNACase after said contacting with a GlcNAc-phosphotransferase.

42. The method of Claim 41, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

43. The method of Claim 42, wherein said lectin is ricin.

5 44. The method of Claim 41, wherein said glycoprotein is a lysosomal hydrolase.

45. The method of Claim 44, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub> Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, 10 Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

46. The method of Claim 45, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

47. The method of Claim 45, wherein the GlcNAc-phosphotransferase comprises 20 SEQ ID NO:2.

48. The method of Claim 45, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.

49. The method of Claim 45, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NOS:4, 5 and 7.

50. The method of Claim 45, wherein the GlcNAc-phosphotransferase is encoded by  
a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence that  
5 hybridizes under stringent conditions to the complement of SEQ ID NO:1.

51. The method of Claim 45, wherein the GlcNAc-phosphotransferase comprises an  
 $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence  
comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under stringent  
conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit, which is encoded  
10 by a nucleotide sequence comprising SEQ ID NO:6 or a nucleotide sequence that  
hybridizes under stringent conditions to the complement of SEQ ID NO:6.

52. The method of Claim 45, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises  
an amino acid sequence of SEQ ID NO:18.

53. The method of Claim 45, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded  
15 by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence  
that hybridizes under stringent conditions to the complement of SEQ ID NO:17.

54. The method of Claim 45, wherein said deoxymannojirimycin is present in an  
amount from about 0.1 mM to about 5.0mM.

55. The method of Claim 45, wherein said kifunensine is in present in an amount  
20 from about 0.1  $\mu$ g/ml to about 10 $\mu$ g/ml.

56. A method of producing a high mannose glycoprotein comprising



- a. a step culturing mammalian cells expressing said high mannose glycoprotein under conditions to produce the high mannose glycoprotein; and
- b. a step for collecting the glycoprotein.

5 57. The method of Claim 56, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

58. The method of Claim 57, wherein said lectin is ricin.

59. The method of Claim 56, wherein said glycoprotein is a lysosomal hydrolase.

10 60. The method of Claim 59 wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub> Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, 15 Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

20 61. The method of Claim 60, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

62. The method of Claim 56, further comprising a step for transferring a N-acetylglucosamine-1-phosphate from UDP-GlcNAc to said glycoprotein.

63. The method of Claim 62, further comprising a step for purifying said glycoprotein comprising a N-acetylglucosamine-1-phosphate.

5 64. The method of Claim 62, further comprising a step for removing an N-acetylglucosamine from said glycoprotein.

65. A high mannose glycoprotein produced by the method of Claim 56.